Toxic Peptides and Amino Acids in Foods and Feeds

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The chemical and physiological properties of some amino acids and peptides found in foods and feeds which have been shown to be toxic to man and domestic animals are reviewed. Compounds discussed include peptides from poisonous mushrooms, lathyrogenic amino acids, hypoglycin A, mimosine, and selenoamino acids.

The discovery that certain contemporaneous organisms were unpalatable or harmful when consumed was probably made very early in the evolution of animals. Man undoubtedly continued this experimental, observational approach to haute cuisine with the result that the palatable, nontoxic organisms, or products derived therefrom, constitute our present day foods and feeds.

Ingestion of the more toxic plants and animals by primitive man or his animals resulted in spectacular acute physiological effects, usually culminating in death. Observing this, he specifically eliminated these very poisonous organisms from his diet. Nevertheless, mistakes either in identification or preparation of these acknowledged toxic organisms result in tragic events even to this day. Thus consumption of poisonous mushrooms inadvertently collected in the wilds accounts for several deaths around the world each year and poisonings resulting from improper preparation of fish are not uncommon in the western Pacific.

Identification of the cause of chronic toxicity on the other hand is much more difficult. The observed symptoms may be nothing more definite than a nonspecific malaise. In general, the effects ascribed to the ingestion of toxic amino acids fall into this category. With the exception of the Amanita peptides, the examples presented in this review are those in which a definite cause and effect relationship has been demonstrated between the consumption of an organism containing a toxic amino acid and the appearance of specific symptoms in the consumer, although the biochemical mechanism of this toxic effect may be unknown.

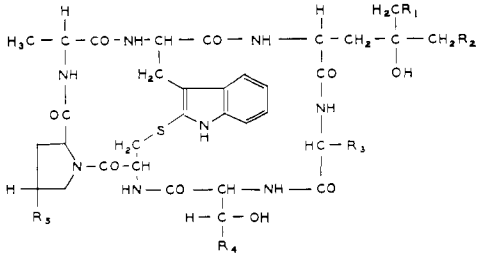
Recently, there have been published a number of excellent reviews on peptides (Waley, 1966) selenium derivatives in proteins (Jauregui-Adell, 1966), toxic amino acids as antimetabolites (Fowden *et al.*, 1967), and nonprotein amino acids (Synge, 1968). The present article is restricted to only those toxic substances found in food and feed.

PEPTIDES

Despite the great hazards involved in such an undertaking, many people persist in collecting and eating wild mushrooms. Expert knowledge of edible and inedible varieties protects the true phycophylls, but the amateurs occasionally make mistakes which usually have a fatal outcome.

Green and white species belonging to the genus Amanita account for approximately 95% of the fatal mushroom poisonings. The most important toxic substances in these mushrooms are peptides whose structures have been elucidated by Theodor Wieland and coworkers (1967, 1968). The peptides may be divided into two groups, the phallotoxins (Figure 1), of which phalloidin shows the typical structure, and the amatoxins (Figure 2), an example of which is α-amanitin.

The phallotoxins are cyclic heptapeptides containing several novel amino acids and unusual combinations of the more common protein amino acids. The most striking structure in the phallotoxins is the thioether, tryptathionine, formed between L-tryptophan and L-cysteine. Reduction of the phallotoxins with Raney nickel in methanol gives the nontoxic dethio derivatives, indicating that the thioether bridge is required for biological activity. Further insight into the structural requirements for toxicity is gained by partial hydrolysis of the phallotoxins in 50 to 80% trifluoroacetic acid at 20° C. This process selectively opens the peptide ring at the carboxyl end of the γ-hydroxylated



PHALLOTOXINS

	R_1	R_2	R_3	R_4	R_5
Phalloidin	OH	H	CH_3	CH_3	OH
Phalloin	H	H	CH_3	CH_3	OH
Phallisin	OH	OH	CH_3	CH_3	OH
Phallicidin	OH	H	$CH(CH_3)_2$	CO_2H	OH
Phallin B (tentatively)	H	H	$CH_2C_6H_5$	CH_3	H

Figure 1. Phallotoxins (redrawn from Wieland (1968))

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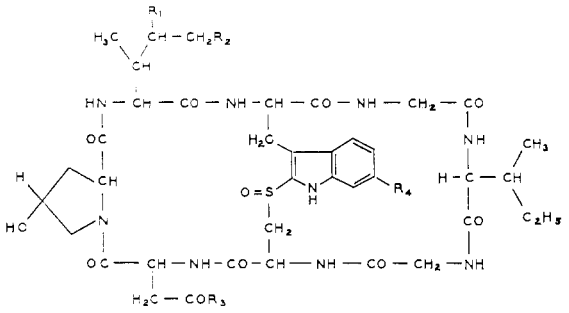

AMATOXINS

	R_1	R_2	R_3	R_4
α-Amanitin	OH	OH	NH_2	OH
β-Amanitin	OH	OH	OH	OH
γ-Amanitin	H	OH	NH_2	OH
Amanin	OH	OH	OH	H
Amanullin	H	H	NH_3	OH

Figure 2. Amatoxins (redrawn from Wieland (1968))

amino acid residues giving rise to nontoxic seco derivatives. Amino acids found in some but not all the members of the phallotoxin group include L-allohydroxyproline, L-proline, D-threonine, L-valine, D-erythrohydroxyaspartate, and L-phenylalanine. L-alanine is the only amino acid common to all phallotoxins. In addition, all the peptides in this group contain γ-hydroxyamino acids derived from L-leucine. The question of the essentiality of the γ-hydroxy function for biological activity has been answered by Wieland (1968), at least for phalloidin. Oxidation of phalloidin with periodate gives the toxic keto derivative. Conversion of this product to the dithiolane, followed by reduction under conditions which do not affect the thioether bridge, produces norphalloidin, which is as toxic as phalloidin. This eliminates the γ-hydroxyl function as a prerequisite structure for toxicity in the phallotoxins.

The amatoxins are octapeptides, and might be considered the next higher homologue of the phallotoxins if it were not that several important structural features set them apart. These peptides, with one exception, contain two glycyl residues, L-aspartic acid or L-asparagine, L-hydroxyproline, L-isoleucine, and various γ-hydroxyisoleucines. The exception is amanullin which does not contain a γ-hydroxyisoleucine and is nontoxic. Thus, contrary to the phallotoxins, where the γ-hydroxyfunction was not required for activity, in the amatoxins this structure is essential for toxicity. In place of the thioether bridge observed in the phallotoxins, amatoxins have been shown to contain a sulfoxide structure (Faulstich *et al.*, 1968) formed between cysteine sulfinic acid and, in all but one case, 6-hydroxytryptophan.

In addition to chemical differences between the two groups of peptides, there are striking differences in their toxic effects. Phallotoxins act quickly and at higher dosages, causing death in 1 to 2 hours. Amatoxins act more slowly and at lower dosages. Increasing the dosage does not reduce the time required for lethal effects to less than 15 hours. α-Amanitin is 10 to 20 times more toxic than phalloidin and thus constitutes the major poison in deadly amanita. The organ affected by both groups of peptides is the liver (Wieland, 1965), but in markedly dissimilar ways. Phalloidin intoxication is associated with changes in the microsomal fraction of the liver cells but only if the compound is administered to the intact animal. This has led to the suggestion that phalloidin itself is not toxic but is converted to the toxic form by the microsomes (von der Decken *et al.*, 1960; Fiume, 1965). Newborn rats with undeveloped drug metabolizing systems tolerate a much higher dose of phalloidin than do adult animals whose microsomal enzyme complement is presumably complete. In addition, destruction of these enzymes in adult animals by administration of carbon tetrachloride results in the tolerance of large amounts of phalloidin before toxicity becomes evident (Floersheim, 1966).

Amatoxins, on the other hand, do not damage cytoplasmic structures at first but rather bring about fragmentation of the nucleoli, the site of RNA synthesis in the nucleus (Fiume and Laschi, 1965). The earliest observed cytological effects occur about 15 hours after administration of α-amanitin. This corresponds precisely with the observed latent lethal period for this substance.

The poisonous species of Amanita contain approximately 10 mg. of phallotoxins and 14 mg. of amatoxins per 100 grams fresh wt. (5 grams dry wt.). Since the toxic dose of amatoxins for the human is less than 100 μg. per kg., it is possible that consumption of one 50-gram mushroom can cause death. The present status of our knowledge of mushroom poisoning is that the toxic effects are caused by a series of cyclic peptides, but that the biochemical mechanism to toxicity is not known.

AMINO ACIDS

The deleterious, naturally occurring amino acids usually produce less spectacular symptoms than the peptide toxins. The toxicity may be manifest in a variety of symptoms but the mechanism of toxicity is usually due to one of the following effects: competitive inhibition of enzymes because of resemblance to the normal substrate; interference in the activation and transfer of the normal amino acid to transfer RNA; interference in the assembly of amino acids into protein and thus interrupting protein synthesis; and incorporation into protein resulting in the formation of defective or nonfunctional proteins.

With the exception of the first effect, these are rather subtle metabolic toxicities and may appear as generalized ill health rather than as dramatic poisoning. Because of this the majority of toxic amino acids discussed in this section are responsible for enzyme inhibition not directly related to protein synthesis.

Lathyrogenic Amino Acids. Seeds of plants belonging to the genus *Lathyrus* have been implicated as the causative agents in the disease complex called lathyrism characterized by damage to the skeletal bonds and the nervous system. Selye (1957) has separated these symptoms into two diseases, osteolathyrism (odoratism), characterized by bone defects, and the neurological disease, neurolathyrism.

The first causative agent was isolated from *L. odoratus* by Dupuy and Lee (1954) and Schilling and Strong (1954) and was identified as γ-glutamyl-β-aminopropionitrile (Figure 3). It was later shown that the β-aminopropionitrile moiety was the actual toxicant (Dasler, 1954). This compound apparently interferes with the cross-linking of collagen. Although only 0.1 to 0.2% of β-aminopropionitrile in the diet of rats is sufficient to produce osteolathyrism, *L. odoratus* has never been associated with lathyrism in humans. The latter malady is endemic in central India and becomes particularly prevalent in times of famine. Under these adverse nutritional conditions *L. sativus*

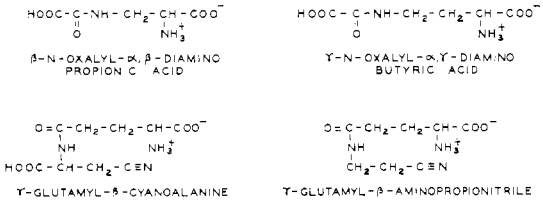

Figure 3. Lathyrogenic and neurotoxic amino acids

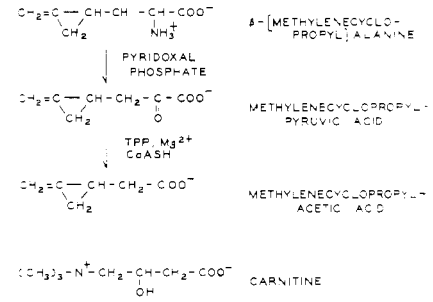

Figure 4. Hypoglycin A, metabolites, and carnitine

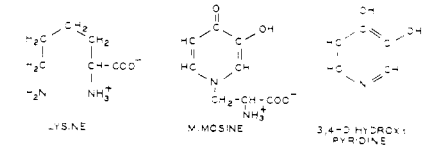

Figure 5. Mimosine and related compounds

(Khesari dal) becomes a major constituent of the diet. A strongly neurotoxic ingredient has been isolated and characterized as β-*N*-oxalyl-L-α,β-diaminopropionic acid (Adiga *et al.*, 1962). Neither oxalic acid nor the free amino acid produces neurological symptoms. Administration to chicks of 20 mg. per kg. of the oxalyl derivative causes neurolathyrism. Bell (1967) and Bell and Tirimanna (1965) have reported the occurrence of this compound in the seeds of 20 species of Lathyrus. In addition, nine species contain the higher homologue, γ-*N*-oxalyl-α,γ-diaminobutyric acid (Bell and O'Donovan, 1966). α,γ-Diaminobutyric acid alone has been shown to be a neurotoxin (Ressler *et al.*, 1961). *Vicia sativa* is usually found in areas where *L. sativus* and other species of Lathyrus are grown and seeds of this plant are found as contaminants in some wheat supplies. A neurotoxic principle has also been isolated from seeds of *V. sativa* and shown to be β-cyano-L-alanine (Ressler, 1962). There is no convincing evidence that this latter compound is involved in human lathyrism and the principal toxic amino acid causing this disease appears to be the oxalyl derivative of α,β-diaminopropionic acid.

Hypoglycin A. The fruit of the tropical tree *Blighia sapida* is a popular food in the Caribbean where it is called "akee" and in Nigeria where it is called "isin." Illness is caused by eating improperly prepared, unripe fruit, and usually occurs when food is scarce as in the winter, or in areas of poverty. The external symptoms include severe vomiting while the principal biochemical sign is an acute hypoglycemia. Individuals suffering from malnutrition or incipient starvation are particularly susceptible, with the mortality in this group being high and usually occurring within 12 hours of the first symptoms. The fruit has been found to contain β-methylenecyclopropylalanine, named hypoglycin A owing to its clinical manifestations. Holt and Holt (1959) and Holt (1966) have proposed that hypoglycin A, or a metabolite, interferes with the β-oxidation of fatty acids for energy. Thus the afflicted individual must metabolize glycogen for energy and rapidly depletes his stores with the resultant hypoglycemia. This agrees with the observation that undernourished individuals, who incidentally have very low glycogen reserves, are the most susceptible to this illness. In later work, methylenecyclopropylacetic acid has been identified as the toxicant and its derivation from hypoglycin A is shown in Figure 4. Recently, Entman and Bressler (1967) have determined that methylenecyclopropylacetic acid interferes with the transfer of long chain fatty acyl residues from coenzyme A to carnitine. β-Oxidation of long chain fatty acids occurs within the mitochondria whose membrane is impermeable to acyl coenzyme A molecules. The membrane is permeable, however, to acyl carnitine molecules and these transport the long chain fatty acyl residues from the cytoplasm to the site of β-oxidation within the mitochondria. β-Methylenecyclopropylacetic acid interferes with the formation of the long chain fatty acyl carnitines and thus blocks β-oxidation. Hypoglycin A has recently been shown to have teratogenic properties when administered to rats by interperitoneal injection (Persaud, 1968). A similar hypoglycemic amino acid, (methylene cyclopropyl) glycine occurs in the seed of *Litchi chinensis* (lychee) but there are no reports of it causing illness in animals since the seed is rarely consumed (Gray and Fowden, 1962).

Mimosine. The toxic amino acid minosine (Figure 5) occurs in legumes belonging to the genera *Leucaena* and *Mimosa* (Brewbaker and Hylin, 1965; Hegarty *et al.*, 1964; Renz, 1936). It is formed from lysine in *Leucaena leucocephala* (Hylin, 1964) and is degraded to 3,4-dihydroxypyridine by soil and rumen microorganisms and by plants (Smith and Fowden, 1968). The most important species is *L. leucocephala* which occurs as a pandemic weed in tropical and subtropical areas. In some countries it is used as a shade tree and hedge for coffee and tea plantings. In other areas it is cultivated as animal feed. This latter use is marginal since diets containing more than 20% *L. leucocephala* usually cause deleterious symptoms in livestock, particularly nonruminants. The symptoms observed include inanition, decreased weight gain, cataracts in young animals (Yoshida, 1944), loss of hair (Crounse *et al.*, 1962; Kostermans, 1946; Renz, 1936) and infertility (Hylin and Lichton, 1965). The causal agent for all these symptoms has been shown to be mimosine which may be present in concentrations as high as 8% on a dry weight basis in young leaves and is typically 3% in older leaves and seeds. The structure of mimosine was determined by Adams *et al.* in 1945, but the precise mechanism of its toxicity remains obscure. It is a low grade inhibitor of the pyridoxal requiring transaminases (Lin *et al.*, 1963), tyrosine decarboxylase (Crounse *et al.*, 1962), and several metal containing enzymes (Chang, 1960; Lin *et al.*, 1963; Tsai, 1961). It is also implicated in tyrosine metabolism in rats since it is asserted that supplementing a mimosine containing diet with tyrosine eliminates the growth retardation normally observed (Lin, *et al.* 1964). Results of studies in our laboratories have not confirmed this latter finding. Recently, we have shown that mimosine is a potent inhibitor of both cystathionine

synthetase and cystathionase from rat liver. Inhibition of the system forming cysteine from methionine is significant since the protein of hair contains an unusually large amount of cysteine. Some of the cysteine in hair is undoubtedly formed from methionine. A decrease in the cysteine available for hair protein synthesis would reduce or even stop hair growth with concomitant loss of preformed hair and wool without regrowth. This is exactly the phenomenon observed by Crounse *et al.* (1962) in experimental animals intoxicated with mimosine.

The possibility that mimosine might be incorporated into protein has been investigated in the rat by Hylin and Lichton (1965) and in mung beans by Smith and Fowden (1968) with negative results in both cases. The latter workers found some indication that mimosine participated in a reaction resembling amino acid activations since pyrophosphate exchange took place. However, they could not detect binding of mimosine to transfer RNA or ribosomal protein. At present, the most satisfactory explanation for the toxicity of mimosine is that it inhibits primarily those enzymes requiring pyridoxal phosphate and specifically the cysteine synthesizing system in rat liver. There also appears to be some inhibition of enzymes requiring heavy metals for catalysis. It should be emphasized that these conclusions are drawn from in vitro experiments and that the true mechanism of mimosine toxicity in vivo has yet to be established.

Amino Acids Containing Selenium. The toxic amino acids discussed up to this point have not been found in proteins. However, the amino acids containing selenium, which occur in a number of cultivated and wild plants growing on seleniferous soils, have been isolated from plant (Trelease *et al.*, 1960) and animal proteins. The most thorough recent survey of the toxicities caused by the seleno amino acids can be found in the text "Selenium" by Rosenfeld and Beath (1964).

Selenium poisoning caused by selenoamino acids is of the chronic rather than acute type. Two disease syndromes of chronic selenium toxicity have been ascribed to organic selenium compounds. The descriptive term "blind-staggers" applied to chronic selenosis occurring in animals consuming native range weeds belonging to the genera *Astragalus* and *Machaeranthera*. The causative agents are believed to be the nonprotein amino acids methylselenocysteine and selenocystathionine (Figure 6). It is uncertain whether these compounds, which occur free in plants, are incorporated into protein by the intoxicated animal.

The second chronic disease of livestock caused by organoselenium compounds is similar to "alkali disease" caused by excessive alkaline salts in soil and water. The disease caused by selenium compounds, however, is clearly distinct. The symptoms, which include loss of hair and deformation and sloughing of hoofs, occur after livestock have grazed for several weeks on grain and forage grasses grown in seleniferous soils. Corn, wheat, barley, oats, grasses, and hay cause the disease when they contain from 10 to 30 p.p.m. of selenium. The selenium in these plants is present in protein in the form of selenomethionine and selenocysteine residues. Digestion of this protein in the digestive tract results in the liberation of these amino acids which are absorbed and distributed throughout the animal by the circulation. Selenomethionine and selenocysteine are biochemically similar to their sulfur analogs and so compete for introduction into protein (Cowie and Cohen, 1957). Selenomethionine inhibits cell division but not cell growth in the green alga *Chlorella vulgaris* (Shrift, 1954). A methionine requiring mutant of *Escherichia coli* could subculture through 100 generations in the presence of selenomethionine without any change in morphology (Cowie and Cohen, 1957). On the other hand, the activity of β-galactosidase synthesized by *E. coli* in the presence of selenomethionine was one-third that observed when methionine was present. This indicates that either less enzyme was synthesized or the specific activity of the enzyme was reduced because of the presence of selenomethionine in the protein. Similar defective proteins resulting from the replacement of the sulfur amino acids by their selenium analogs could account for the loss of hair and sloughing of hoofs described in "selenium alkali disease."

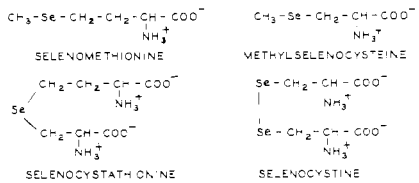

Figure 6. Selenoamino acids

Since these selenoamino acids are found in cereal proteins it is possible that humans eating cereals grown in seleniferous soils would be similarly affected. Chronic selenosis in humans presumably caused by eating corn grown in seleniferous soil has been reported from Columbia, South America (Rosenfeld and Beath, 1964). No similar example is available from North America although Smith *et al.* (1936), Smith and Westfall (1937), and Smith and Lillie (1940) attempted to correlate selenium in the food of rural populations living in South Dakota and Nebraska in areas with seleniferous soils with disease symptoms specific for selenium toxicity. Among the members of 111 families studied, there appeared to be higher than normal incidences of yellowish skin, dermatitis, chronic arthritis, bad teeth, and diseased nails on fingers and toes. In general, a majority of the individuals afflicted with one or more of these symptoms had a daily urinary excretion of 0.20 p.p.m. or greater of selenium.

Whether selenoamino acids present in foods produced in seleniferous areas are responsible for the rather vague symptoms encountered in these studies is not known. Milk, eggs, and meat produced in these seleniferous areas by animals fed grain or pasture grasses grown in the same areas had selenium contents which ranged from a trace to 17.8 p.p.m.

SUMMARY

The vast majority of foods and feeds consumed by man and his domesticated animals does not contain toxic amino acids and peptides. Those food items which do contain these toxicants are usually unique to certain limited climatic areas of the world and are consumed by relatively small populations in these areas. The mechanism of toxicity of the amino acids is generally inhibition of specific enzyme catalyzed reactions. The peptide toxins on the other hand appear to destroy cellular integrity although their precise mode of action is not known and may in the future be shown to be specific enzyme inhibition. Selenomethionine and selenocysteine appear to be incorporated

into protein and thus result in altered secondary or tertiary structures which affect enzyme activity and other biological properties. Toxic amino acids and peptides are also present in nonfood plants but these do not fall within the purview of this article. Fowden *et al.* (1967) and Waley (1966) present information on these compounds.

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